Remarks

The specification has been amended to correct minor typographical errors therein. Support for the amendments can be found throughout the subject specification, including, for example, at page 15, lines 11-12 and line 22. For example, it is clear that reference to "Bam HI" at page 16 should read as "Sma I" in view of the reference to the plasmid pSwick-BXWT having a Sma I restriction site. Entry of the amendments presented herein is respectfully requested. Applicant respectfully asserts that no new matter has been added by these amendments.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,

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DRP/sl
Attachments: Marked-Up Version of Substituted Paragraphs; Abstract of the Disclosure

Marked-Up Version of Substituted Paragraphs

Paragraph on page 15, beginning at line 14:

First, a DNA fragment containing CFTR amino acids [T351-F492] T351-S492 was produced using pSwick-BXWT plasmid DNA as template and the primers PRNBD1-R1 (5'-CGCGGAATTCACTCGGCAATTTCCC-3') (SEQ ID NO:1) and PRNBD1-PST (5'-GCGCCTGCAGTTAAGAACAGAATGAAAT-3') (SEQ ID NO:2) in the polymerase chain reaction (PCR). The resulting [142] 449 bp DNA fragment contained an Eco R1 restriction endonuclease site preceding the CFTR amino acid T351 and a Pst I site following CFTR amino acid S492. The fragment was restricted with EcoR1 and Pst I restriction endonucleases, and ligated into the unique Eco RI and Pst I restriction sites within pAD-GAL4 to produce pADPRNBD1 in which CFTR amino acids [are] T351-S492 are joined in frame to the pAD-GAL4 transcription activation domain. A second GAL4-CFTR fusion plasmid was constructed in which a 951 bp HpaI-TaqI DNA fragment from pSwick-BXWT (containing CFTR amino acids R334-F650, and with the ends of the fragment made blunt by [kenow] klenow fragment) was purified from an agarose gel and ligated into the Sma I site of plasmid pBDGAL4 to produce PBD-N. The pBD-N plasmid DNA was then cut with Eco RI and Bam HI and the vector molecule purified from an agarose gel. The purified Eco RI-Bam HI pBD-N vector molecule was then ligated to an Eco RI- Bam HI restriction fragment from pADPRNBD1 (containing amino acids T351-S492 of CFTR), producing pBDPN-WT. The pBDPN-WT plasmid contains CFTR amino acids T351-F650 fused in frame to the GAL4 DNA binding domain. This region contains the predicted cytosolic region that precedes NBD1, the NBD1 region, and also a segment that had previously been ascribed to the R domain. The plasmid pBDPN-WT also contains the TRP1 gene of yeast, and replication origin from the yeast 2μ plasmid. The Eco RI-Pst I fragment from pBDPN-WT (containing CFTR amino acids T351-F650) was then cloned into the EcoRI and Pst sites of pADGAL4, producing pADPN-WT. pADPN-WT contains CFTR amino acids T351-F650 fused in frame to the GAL4 activation domain. The pADPN-WT plasmid also contains the LEU2 gene of yeast and the replication origin of the yeast 2μ circle. Both plasmids pBDPN-WT and pADPN-WT were introduced by transformation into yeast cell strain YGR-2 to produce cells designated as YRG2-WT.

Paragraph on page 16, beginning at line 11:

A plasmid identical to pBDPN-WT, but containing the ΔF508 mutation (pBDPNΔF) was constructed by cutting pBDPN-WT with [Bam HI] Sma I and Xho I, and replacing the approximately 180 bp [Bam HI-Xho I] Sma I-Xho I fragment (containing the wildtype CFTR region P499-R560) with the corresponding [Bam HI-Xho I] Sma I-Xho I fragment from pSwick-BXΔF containing the ΔF508 mutation. Similarly, a plasmid identical to pADPN-WT, but containing the ΔF508 mutation was constructed. Both plasmids pBDPN-ΔF and pADPN-ΔF were introduced by transformation into yeast cell strain YGR-2 to produce cells designated as YRG2-ΔF.